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Rapid Detection of Hydrogen Peroxide Based on Aggregation Induced Ratiometric Fluorescence Change

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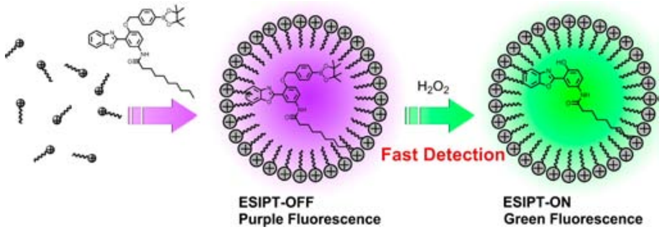
ABSTRACT



In surfactant solution, probe D-BBO can detect H_2O_2 with an enhanced reaction rate ($k_{obs} = 1.83 \times 10^{-2}\ s^{-1}$) and a large bathochromic shift of 105 nm. Furthermore, D-BBO displays a highly selective response to H_2O_2 over other reactive oxygen species under identical conditions.

Fluorescent sensing is a very important research topic of supramolecular chemistry and provides an efficient method for analyte reporting.[1] Hydrogen peroxide (H_2O_2) is a common chemical and widely used for bleaching, cleaning, and disinfection.[2] It is also an important biological molecule connected to many physiological processes, such as oxidative damage, defense response, and cellular signal transduction.[3] Owing to the development of molecular probes in the past few years, fluorescent visualizing and monitoring of H_2O_2 has been realized with high sensitivity and selectivity.[4] Up to now, most of these probes are designed according to a chemical-reaction-based approach, which takes advantage of a specific chemical group, such as benzenesulfonyl ester, benzil, or boronate ester to react with H_2O_2.[4,5] However, these reactions mostly need a long reaction time to achieve signal output, which may lead to a potential influence on real-time monitoring and quantification. Therefore, it is still challenging to design efficient probes that can rapidly respond to H_2O_2.

It is well-known that the amphiphilic compounds, consisting of a hydrophilic headgroup and a hydrophobic tail,

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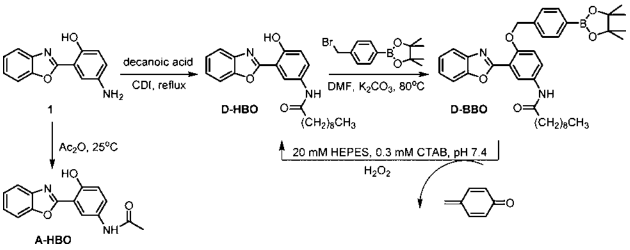
can form micellar systems and offer a microenvironment to accelerate the observed rates of some chemical reactions.[6] Molecular recognition and fluorescent sensing can greatly benefit from the micellar systems as well.[7] However, fluorescent dyes or probes in the micelles may tend to aggregate, generally leading to fluorescence quenching and reduction of probe sensitivity.[8,9] Thus, the photophysical properties of the fluorophore need to be further elaborated. We speculate that the combination of a traditional fluorescent sensing mechanism with the surfactant effect could set up an efficient and simple method for rapid detection of H_2O_2.

Our strategy is to choose 2-(2′-hydroxyphenyl)benzoxazole (HBO) as a fluorophore and C9 alkyl chain as the hydrophobic tail (Scheme 1). HBO analogs are classic fluorescent compounds, exhibiting a unique aggregation-induced emission enhancement (AIEE) phenomenon.[10] In the aggregated form, the intramolecular rotation is restricted, resulting in the restriction of the nonradiative pathway, so that they mostly exhibit an intensely pronounced excited-state intramolecular proton transfer (ESIPT) emission resulting from the keto form.[11] In present paper, the hydroxyl group was protected with the boronate-based benzyl cleavable group to obtain probe D-BBO based on the following considerations: (i) increasing the hydrophobicity of D-BBO, (ii) inhibition of the ESIPT process, and (iii) specificity toward H_2O_2. We envisioned that probe D-BBO can easily aggregate into particles in a surfactant solution and emit purple fluorescence due to the absence of ESIPT. Under the surfactant acceleration, the H_2O_2-triggered boronate cleavage would take place more rapidly and liberate the ESIPT compound D-HBO, giving rise to considerable red-shifted fluorescence emission. Therefore, a rapid detection of H_2O_2 with ratiometric signal output can be established.

Scheme 1. Synthesis of Compounds



The synthetic procedures are shown in Scheme 1. Compound D-HBO can be easily prepared by coupling compound **1** with decanoic acid. The hydroxyl group was further alkylated by using 4-(bromomethyl)benzene boronic acid pinacol ester under basic conditions giving D-BBO in moderate yield. A control molecule with a short alkyl chain, A-HBO, was also synthesized by direct acetylation of compound **1**.

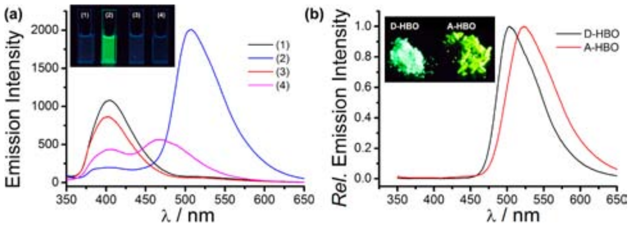


Figure 1. (a) Fluorescence spectra of 4 μM D-HBO in DMSO (1); H_2O/DMSO = 1:1 (2); 4 μM A-HBO in DMSO (3); H_2O/DMSO = 1:1 (4), λ_{ex} = 341 nm. Inset: photographs of the solutions, λ_{ex} = 365 nm. (b) Fluorescence spectra of D-HBO and A-HBO in solid state. Inset: photographs of the solids, λ_{ex} = 365 nm.

As shown in Figure 1a, both D-HBO and A-HBO exhibit fluorescence emission around 405 nm in DMSO, which strongly breaks the intramolecular hydrogen bond leading to the inhibition of the ESIPT process.[12] In a mixed solution (H_2O/DMSO = 1:1), the emission peak of D-HBO is obviously red-shifted, whereas A-HBO shows fluorescence quenching with a much smaller emission shift. The distinct emission color of solutions can be easily observed by the naked eye (Figure 1a, inset), whereas both D-HBO and A-HBO exhibit strong fluorescence around 500 nm in the solid state (Figure 1b). This phenomenon clearly suggests that these features are assignable to the AIEE mechanism. However, the water molecules can induce the aggregation of hydrophobic D-HBO molecules, which reduce the fluorescence quenching caused by the

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intramolecular rotation. As a result, D-HBO exhibits apparent ESIPT emission in H_2O/DMSO solution.[10,11]

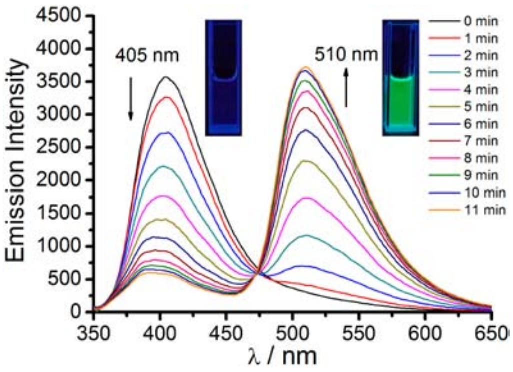

Figure 2. Fluorescence emission spectra of D-BBO (4 μM) upon addition of 100 μM H_2O_2 for 0−11 min in HEPES buffer (20 mM HEPES, 0.3 mM CTAB, pH = 7.4, 25 °C), λ_{ex} = 341 nm.

Boronate-caged probe D-BBO is highly hydrophobic and its fluorescence emission gradually decreased within 60 min in aqueous solution (Figure S1a). But the fluorescent intensity of D-BBO displayed almost no change during the same period in the cetyltrimethylammonium bromide (CTAB) aqueous solution (20 mM HEPES, 0.3 mM CTAB, pH = 7.4) (Figure S1b), suggesting that the probe can be well dispersed in this solution. As shown in Figure 2, D-BBO exhibits a strong fluorescence emission maximum at 405 nm ($\Phi = 0.21$), which is very similar to the enol form emission of D-HBO in DMSO.[13,7i] Addition of 100 μM H_2O_2 induces a rapid decrease of the emission at 405 nm and concomitantly a dramatic increase of the emission at 510 nm ($\Phi = 0.37$) with a large bathochromic shift of 105 nm and an isoemissive point at 474 nm. These data clearly suggested that H_2O_2 triggered the boronate cleavage process so as to generate D-HBO, which emits ESIPT fluorescence. This process was also confirmed by the ESI-MS analysis (Figure S3). As expected, the probe showed a rapid optical response to H_2O_2. Using pseudo-first-order kinetics (2 μM D-BBO, 1 mM H_2O_2), the observed rate constant for H_2O_2-deprotection is $1.83 \times 10^{-2}\ s^{-1}$, which is 20−60-fold greater than that of reported H_2O_2-selective probes.[4c,d] Thus, D-BBO can be an excellent ratiometric probe for rapid detection of H_2O_2 in micellar systems with a large emission shift and ratio changes.

With these data in hand, we next investigated the effect of CTAB on the rates of detection. It is well-known that CTAB is a cationic surfactant and widely used to construct micellar systems for catalyzing chemical reactions. The critical micelle concentration (CMC) is 0.9 mM in water.[14] To further evaluate the acceleration effect of micelles, we prepared a series of surfactant solutions with various CTAB concentrations (0.1−2.0 mM). The observed rate constants of D-BBO for H_2O_2 were measured and shown in Figure 3. The maximum value of $1.83 \times 10^{-2}\ s^{-1}$ is found at the CTAB concentration of 0.3 mM. Under these conditions, the CTAB molecules do not form micelles. However, while the hydrophobic D-BBO was added into the CTAB solution, the probe could be encapsulated by CTAB molecules via the interactions of long alkyl chains, resulting in formation of well dispersed cationic particles, which was further confirmed by dynamic light scattering (DLS) measurements (Figure S4). The aggregated particles have an average size of about 195 nm when the concentration of CTAB is 0.3 mM, which is apparently larger than that in 2 mM CTAB solution (105 nm). In our system it turns out that 0.3 mM CTAB solution has the best acceleration effect on H_2O_2-sensing, probably because of the formation of aggregate with appropriate size and charge distribution under these conditions. Since the boronate cleavage highly depends on the nucleophilicity of H_2O_2,[4k] the cationic particles can obviously accelerate the reaction of boronate substrate with anionic HOO^- through an electrostatic effect, as observed in other micelles systems.[6] The aggregation of product D-HBO restricts intramolecular rotation, which is beneficial to the intramolecular hydrogen bond, and consequently turns on the ESIPT emission (Figure 4). Additionally, neither anionic sodium dodecylsulfate (SDS) nor nonionic Triton X-100 shows the acceleration effect on the boronate cleavage (Figure S5).

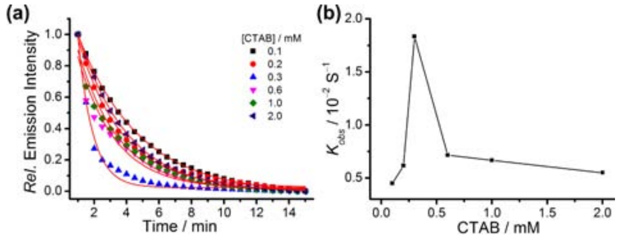

Figure 3. (a) Time-course kinetic measurement of fluorescence response of D-BBO to H_2O_2. Data were collected under pseudo-first-order conditions (2 μM D-BBO, 1 mM H_2O_2) in HEPES buffer (0.1−2.0 mM CTAB, λ_{ex} = 341 nm, 25 °C, λ_{em} = 405 nm). Data collected from 1 to 15 min were used to fit the rate constants (red lines). (b) The observed rate constant change as a function of CTAB concentration.

The boronate group has been widely introduced to design fluorescent probes for detection and imaging H_2O_2 by Chang and other groups.[4] The most notable feature of these probes is the high selectivity to H_2O_2 over other reactive oxygen species in aqueous solutions. In order to check whether this chemoselectivity could be preserved under surfactant conditions, we further performed fluorimetric titration of D-BBO with different reactive oxygen species (ROS) for 10 min in surfactant buffer (20 mM HEPES, 0.3 mM CTAB, pH = 7.4). As shown in Figure 5, only H_2O_2 induced a sharp and rapid response in both fluorescence emission and the emission

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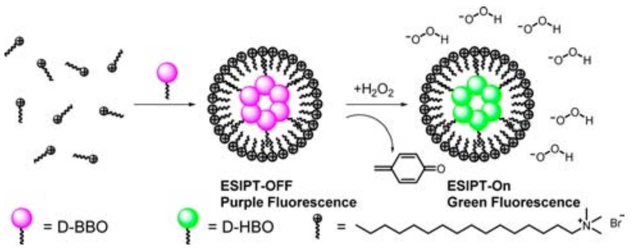

Figure 4. Proposed mechanism for rapid detection of H_2O_2.

ratio ($F_{510}/F_{405\ \mathrm{nm}}$). But other ROS, including *tert*-butylhydroperoxide (TBHP), hypochlorite (ClO^-), hydroxyl radical ($\cdot$OH), *tert*-butoxy radical ($\cdot O^tBu$), nitric oxide (NO), and singlet oxygen ($^1O_2$), gave out no obvious fluorescence emission ratio changes. Therefore, we conclude that our probe bears excellent chemoselectivity for H_2O_2 over other competing ROS in surfactant solution.

In conclusion, we designed a new ratiometric fluorescent probe for rapid detection of H_2O_2 in surfactant buffer solution. The probe can be well encapsulated by surfactant molecules leading to cationic aggregates, which apparently accelerate the reaction rate of the probe with H_2O_2. Moreover, the unique AIEE and ESIPT photophysical properties of the boronate-leaved product provide a remarkable fluorescence signal output with an extremely large emission red shift in the sensing process. Therefore, we have established a new method for rapid detection of H_2O_2 based on aggregation induced ratiometric fluorescence change. We also emphasize that this method can be capable of facility and efficiency in the design of reaction-based probes for other species.

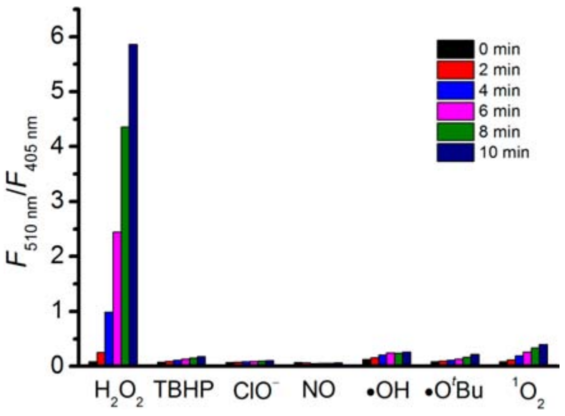

Figure 5. Fluorescence responses of 4 μM D-BBO to various reactive oxygen species (ROS) at 100 μM. Bars represent emission intensity ratios $F_{510}/F_{405\ \mathrm{nm}}$ at 0, 2, 4, 6, 8, 10 min after addition of each ROS. Data were acquired in HEPES buffer (20 mM HEPES, 0.3 mM CTAB, pH = 7.4, 25 °C, λ_{ex} = 341 nm).

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Supporting Information Available. Synthetic procedures, characterization of compounds, and additional spectroscopic data. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.